Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4+ T Cell-Independent" ntors: Qian Huang, et al. T2-K b +SIINFEKL peptide (e.g., 1 x $^{10^{-7}\text{M}}$) Effector Cells elicited with: 120:1 E:T Ratio ■ OVA.TBhsp70 80:1 Т2-Кр Target Cells: CD4-/-Figure 1B ∆ OVA USYCHESH CINCL 120:1 120:1 C57BL/6 (Wild Type) E:T Ratio Figure 1C 80:1 80:1 Figure 1A 82m-/-40:1 20. 40 60 60 40 20

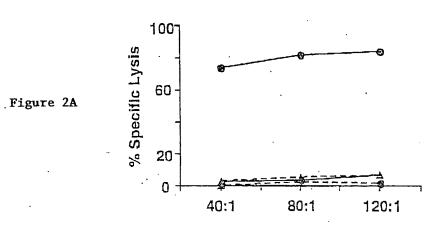
% Specific Lysis

% Specific Lysis

Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4* T Cell-Independent"

Inventors: Qian Huang, et al.

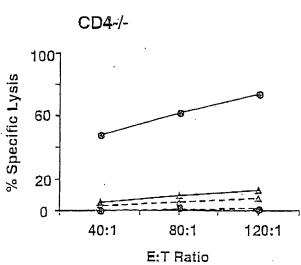
C57BL/6 (Wild Type)



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Figure 2B



Effector Cells Target Cells:
elicited with:
OVA.mhsp70 -- T2-Kb

T2-Kb+SIINFEKL

Inventors: Qian Huang, et al.

Hsp70 Domains

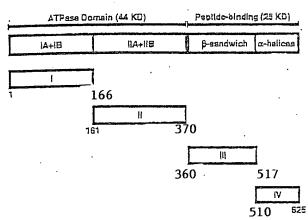


Figure 3

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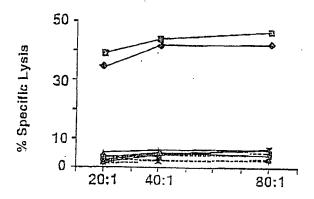
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Docket/App No.: 0399.2006-003

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4* T Cell-Independent"

Inventors: Qian Huang, et al.

C57BL/6



Target Cells:

T2-Kb

T2-Kb+SIINFEKL

Effector Cells elicited with:

■ OVA.TBhsp70

∇ OVA.TBhsp I

OVA.TBhsp II

OVA.TBhsp70 III

OVA.TBhsp70 IV

OVA Δ

Figure 4

 $\begin{array}{ccc} \text{IKVSGLEQLESIYRYYGLLLKEAY} \\ \text{Ova} & & \uparrow & & \uparrow & \alpha \text{KG} \end{array}$

Figure 5A

Hsp65

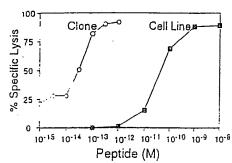
Figure 5B

Vector

Figure 5C

Vector CD69

Figure 5D



Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Protein to a Discrete Domain and is CD4* T Cell-Independent"

Inventors: Qian Huang, et al.



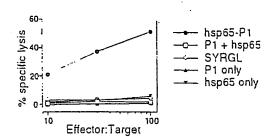


Figure 6B

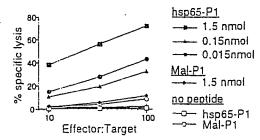
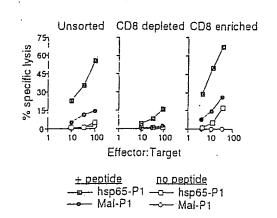


Figure 6C

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Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4⁺ T Cell-Independent"

entors: Qian Huang, et al.

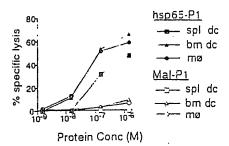
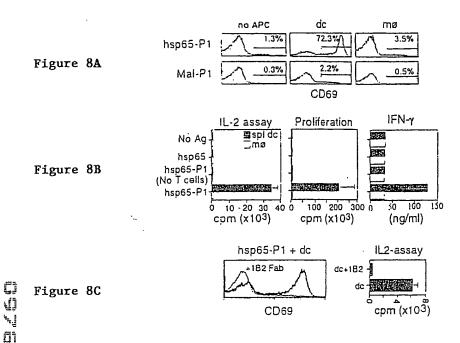


Figure 7

Docket/App No.: 0399.2006-003

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4+ T Cell-Independent"

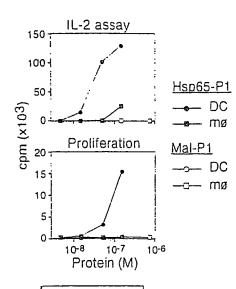
tors: Qian Huang, et al.



Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4* T Cell-Independent"

tors: Qian Huang, et al.







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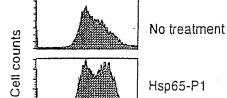
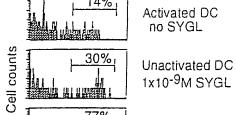
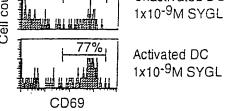


Figure 9C



MHC Class I



Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4* T Cell-Independent"

nventors: Qian Huang, et al.

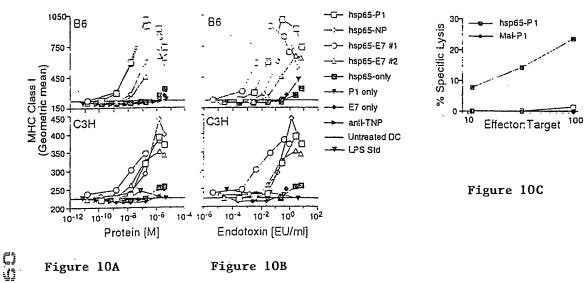


Figure 10A

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Figure 10B

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Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4* T Cell-Independent"

sentors: Qian Huang, et al.

TBhsp70 (cDNA) -> Translate • 1-frame

DNA sequence 1979 bp ATCGCTCGTCCG ... ACGCCAAGTGAC linear

31/11 ATO GOT COT COO GTO GOO ATO GAD CTC GOO AAC TOO GTO GTO TOO GTO GTO MARAVGIDLETTNS 91/31 51/21 GGT GGG GAG COG GTC CTC CCC AAC TCC GAG GGC TCC AGG ACC ACC CCG TCA ATT GTC V V A N S E G S R T T P S 121/41 151/51 SCS THE SEC SEC AAS SET SAR STO STO STO GES SAS SEC AAS AAS SAS GEA GES ACC A F A R N G E V L V G Q P A K N Q A 161/61 211/71 ARC GTC GAT CGC ACC GTG CGC TCG GTC AAU CGA CAC ATG GGC AGC GAC TCG TCC ATA GAG D R T V R S V K R H H C 271/91 ATT GAC GGC AAG AAA TAC ACC GCG CCG GAG ATC ACC GCC ATT CTG ATG AGG CTU AAG рсккута ре I E A R I L M 331/111 CGC GAC GCC GAG GCC TAC CTC GGT GAG GAC ATT ACC GAC GCG GTT ATC ACG ACG CCC GCC RDAEAYLGEDIT I V A C 391/131 361/121 THE THE HAT SHE GET CAG GET CAG GET ACC HAG GHE GET GET CHE ATO GET CHE HAT. T K Q A Q K Y K D A ¢ Q I A G 451/151 421/141 GTG CTG CGG ATC GTC AAC GAG CCG ACC GCG GCC GCG CTG GCC TAC GGC CTC GAC AAG GGC ጥ ል RIVNEP Α а а 511/171 ø r77 Ĩ. 481/161 GAG ANG GAG CAG CGA ATC CTG GTC TTC GAC TTG GGT GGT GGC ACT TTC GAC GTT ICC CTG Z I L ø E71/191 541/131 CTO GAG ATC GGC GAG GGT GTG GTT GAG GTC CGT GGC ACT TCG CGT GAC AAC CAC CTC GGC LEIGEGVVEV RAT S G D M H 501/201 631/211 GGC GAC GAC TGG GAC CAG COG GTC GTC GAT TGG CTG GTG GAC AAC TTC AAG GGC ACC AGC V D K F K G T D W D Q R V V D W D 661/221 691/231 bed ard dar otto acc ard gru ard att ded att cad det ett cod gra ged dec ged gra rag I D L T K D R M A M Q R L R E A 11/241 751/251 731/241 SCA AAG ATC GAG CTG AGT TCG AGT CAG TCC ACC TCG ATC AAC CTG CCC TAC ATC ACC GTC A K I E L S S Q S T S T N P 811/271 791/251 GAC GCC GAC AAG AAC CCG TTG TTC TTA GAC GAG CAG CTG ACC CGC GCC GAG TTC CAA CGG DKNPLFLD E Q L T R D A 871/291 ATC ACT CAG GAC CTG CTG GAC CGC ACT CGC AAG CCG TTC CAG TCG GTG ATC GCT GAC ACC QDLLDRTRRFQS 931/311 901/301 OGU ATT TOU GTG TOG GAG ATC GAT CAC GTT GTG CTC GTG GGT GGT TOG ACC CGG ATG CCC в х з в в р н 961/321 991/331 1021/341 1051/351 DOD GAT GAG GTT GTD GCG GTG GGA GCC GCT CTG CAG GCC GGC GTC CTC AAG GGC GAG GTG v G A A L Q AGVL ANA GAC GIT CIG CIG CIT GAT GIT ACC COG CIG AGC CIG GGT ATC GAG ACC AAG GGC GGG r r Ÿ <u>.. T</u> S L G I E 1171/391 11-1/281 STG ATG ACC AGG CTC ATG GAG CGC AAC ACC ACG ACC ACG AGG CGG TCG GAG ACT TTC NTTIP 1231/411 1201/401 ACC ACC GCC GAC GAC AAC CAA CCG TCG GTG CAG ATC CAG GTC TAT CAG GGG GAG CGT GAA T 1 A D D M 2 F F V Q I 2 V Y Q G E R E A D

Figure 11

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Docket/App No.: 0399.2006-003

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps

to a Discrete Domain and is CD4+ T Cell-Independent"

Inventors: Qian Huang, et al.

TBhsp70 (cDNA) -> Translate • 1-frame

DNA sequence 1879 bp ATGGCTCGTGCG ... AGGCCAAGTGAC linear

```
511/171
EAG AAG CAG CAG CGA ATC CTG GTC TIC GAC TTG GGT GGT GGC ACT TTC GAC GTT TCC CTG
   K E Q R I L Y F D L C O O
                                    571/191
541/191
CTO GAO ATO GGO GAO GGT GTO GTT GAG GTC CGT GCT ACT TCG CGT GAC AAO CAO CTO GGO
LEIGEGVVEV
                                   R A T
                                              S
                                                 G
                                                    D M H L
                                    631/211
501/201
ONG GAC TWO GAC CAS CES OTC GTT GAT TWO CTG GTG GAC AAG TYC AAG GGC ACC AGC
G D D W D Q R V V D W T
                                          V D K F K G T S
651/221
                                    691/231
bec are gar ere ace and gar and are edg are cag cod ctc cod gar ecc ccc gag and b i d d t r d r m a m q r l r b a a b r 751/251
GCR AAG ATC GAG CTG AGT TOG AGT CAG TOC ACC TOG ATC AAC CTG CCC TAC ATC ACC GTC
   K I E L S S Q S
                                   TSINLPI
791/251
                                    811/271
GAC GCC GAC AAG AAC CCG TTG TTC TTA GAC GAG CAG CTG ACC CGC GCG GAG TTC CAA CGG
  A D K N P L F L D
                                   EQLT
                                                 R A E F
                                    £71/291
ATC ACT CAG GAC CTG CTG GAC CGC ACT CGC AAG CCG TTC CAG TCG GTG ATC GGT GAC ACC
                                    931/311
901/301
GOVERTY TOO GTG TOO GAG ATC GAT CAC GTT GTG CTC GTG GGT GGT TCG ACC CGG ATT CCC
      e v s e i d h v
                                    991/331
961/321
GOG GTG ACC GAT CTG GTC AAG GAA CTC ACC GGC GAC AAG GAA CCC AAC AAG GGC GTC AAC
A V T D L V K E L T G G K E P M K G V M
                                                       ⊼
                                    ICS1/351
1021/241 -
CCC GAT GAG GTT GTC SCG GTG SGA GCC GCT CTG CAG GCC GCC CTC CTC AAG GGC GAG GTG
                                   L Q A G V L K G
             V A V
  D
                       G A A
1081/361
ANA GAO GTT DIG CTG CTT GAT GTT ACC CCC
x : 5 v & 5
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Figure 12

Docket/App No.: 0399.2006-003

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps

to a Discrete Domain and is CD4+ T Cell-Independent

Inventors: Qian Huang, et al.

murine hsp70.1 -> Translate • 1-frame

DNA sequence 1929 bp ATGGCCAAGAAC ... GAGGTCGATTAG linear

31/11 ATG GCC AAG AAG ACG GCG ATC GGC ATC GAG CTG GGC ACC ACC TAC TCG TGC GTG GGC GTG MARNTAIGIDLGTTYSCV 97/31 61/21 TTC CAG CAC GGC AAG GTG GAG ATC ATC GCC AAC GAC CAG GGC AAC CGC ACG ACC CCC AGC F Q H G K V E I I A N D Q G N R T 151/51 121/41 THE GTG GCC TTC ACC GAC ACC GAG CGC CTC ATC GGG GAC GCC AGG AAG AAC CAG GTG GCG I G D A A R N Q V Y V A F T D T E R 211/71 181/61 CTG AAC CCG CAG AAC ACC GTG TTC GAC GCG AAG CGG CTG ATC GGC CGC AAG TTC GGC GAT A K R L I GR LNPQNTVF D 271/91 241/81 GCG GTG GAG TOO GAG ATG AAG CAC TGG CCC TTO CAG GTG GTG AAC GAC GGC GAC AAG Y N D G D K AVVQSDMKHWPFQV 331/111 301/101 CCC AAG GTG CAG GTG AAC TAC AAG GGC GAG AGC CGG TCG TTC TTC CCG GAG GAG ATC TCG FFPE K V Q V N Y K -O E S .R S 391/131 TCC ATG GTG CTG ACG AAG ATG AAG GAG ATC GCT GAG GCG TAC CTG GGC CAC CCG GTG ACC YLGHPV S M V L T K M K E I A E A 451/151 421/141 AAC GCG GTG ATC ACG GTG CCC GCC TAC TTC AAC GAC TCT CAG CCG CAG GCC ACC AAG GAC V P A Y F N D S Q R Q A T K NAVIT 511/171 481/161 . GCG GGC GTG ATC GCC GGT CTA AAC GTG CTG CGG ATC ATC AAC GAG CCC ACG GCG GCC G V I A G L N V L R I I N E F T 571 /101 541/181 ATC GCC TAC GGG CTG GAC GGG ACC GGC AAG GGC GAG CGC AAC GTG CTC ATC TTC GAC CTG I A Y G L D R T G KGER ΛΓ 631/211 501/201 FIGG GGC GGC ACG TTC GAC GTG TCC ATC CTG ACG ATC GAC GAC GGC ATC TTC GAG GTG AAG I L T I D D G I F E G G G T F D V S 691/231 661/221 SCC AC3 SCS GGC GAC ACS CAC CTG GGA GGG GAG GAC TTC GAC AAC CGG CTG GTG ACC CAC A T A G D T H L G G E D F D N R 751/251 721/241 TTO GTG GAG GAG TTO AAG AGG AAG CAC AAG AAG GAC ATO AGC CAG AAC AAG CGC GCG GTG $\begin{smallmatrix} F & V & E & E & F & K & R & R & R & R & R & D & I \\ \end{smallmatrix}$ R A 811/271 781/261 CGG CGG CTG CGC ACG GCC TGT GAG ACG GCC AAG ACG CTG TCG TCC AGC ACC CAG GCC L S S S T RRLRTACERAKRT 871/291 841/281 AGO CTG GAG ATO CAC TOT CTG TTC GAG GGC ATO GAC TTC TAC ACA TOO ATO ACG CGG GCG S L S I D S L F E G I D F '-Y T 931/311 CCG TTC GAA GAG CTG TGC TCG GAC CTG TTC CGC GCC ACG CTG GAG CCC GTG GAG AAG GCC SPASK 991/331 CTG CGC GAC GCC AAG ATG GAC AAG GCC CAG ATC CAE GAC CTG GTG CTG GTG GGC GGC TCG 361/321 LRDAKMOKAQIHDLVLVG 1051/351 1021/341 ACG CGC ATC CCC AAG GTG CAG AAG CTG CTG CAG GAC TTC TTC AAC GGG CGC GAC CTG AAC RIPKVQKLLQDF 1111/371 AAG AGG ATG AAG CCG GAG GCG GTG GCC TAC GGG GCG GCG GTG CAG GCC ATC CTG PDEAVAYGAAV Q A A 1171/391 1141/391 STG GGG GAC AND TOS GAG AND GTG CAG GAC CTG CTG CTG CTG GAC GTG GCG GGG CTG TCG Q D L L L D V A v v 1231/411 ONG GRO ONG GAG ACT GOO GGO GGO GTG ATG ACG GCG CTC ATG AAG CGC AAC TOU NOO ATG I K R N S LGLETAGGVM m A L 1291/431 1251/421 DOC ACC AAG CAG ACG CAG ACC TYC ACC ACC TAC TOG GAC AAC CAG CCC CGD CTG CTG ATC Y S D N Q P G V L I TRQTQT T T

Docket/App No.: 0399.2006-003
"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps to a Discrete Domain and is CD4⁺ T Cell-Independent" ventors: Qian Huang, et al.

murine nsp/0.1 -> Translate • 1-frame

```
1351/451
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                              R D N
  VYEGERAM
                          T
                              1411/471
CTG AGC GGC ATC CCG CCG GCG CCC AGG GGC CTG CCG CAG ATC GAG GTG ACC TTC GAC ATC
                                      I E A L
                              A b Ö
                        R G
L S G I P P A P
                              1471/491
 T DK
 D Y M C I F M A
                        A T
                              1531/511
 ATC ACC AND ACC AAC GAC AAG GGC CGC CTG AGC AAG GAG GAG ATC GAG CGC ATG GTG CAG
                                       E I E R M V Q
                              SKE
      I I N D K G
                               1591/531
 GAG GCC GAG CGC TAC AAG GCC GAG GAC GAG GTG CAG CGC GAC AGG GTG GCC GCC AAG AAC
                                       D R V A A K N
            YKAEDEVQR
 E A E R
TIGGG CTC GAG TCC TAT GCC TTC AAC ATG AAG AGC GCC GTG GAG GAC GAG GGT CTC AAG GGC
            Y A F N M K S A V E D E G L K
 A L E
                               1711/571
THANG CTC AGC GAG GCT GAC AAG AAG GTC CTG GAC AAG TGC CAG GAG GTC ATC TGC TGG
EK L S E A D K K K V
                                       CQEVI
                              r b k
                               1771/591
TICTO GAC TOO AAC ACO CTG GCC GAC AAG GAG GAG TIC GTG CAC AAG CGG GAG GAG CTG GAG
                              EFVHKREELE
         N T L A D K E
1831/611
1831/611
1801/601
1801/601
CGG GTG TGC AGC CCC ATC ATC AGT GGG CTG TAC CAG GGT GCG GGT GCT CCT GGG GCT GGG
                                          G A P G
                              y Q G
         SPIISGL
GGC TTC GGC CCC CAG GCG CCC AAA GGA GCC TCT GGC TCA GGA CCC ACC ATC GAG GAG
 G F G A Q A P P K
F 1921/641 ·
T GTG GAT TAG
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Figure 13B

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Docket/App No.: 0399.2006-003

"In Vivo CTL Elicitation by Heat Shock Protein Fusion Proteins Maps

to a Discrete Domain and is CD4+ T Cell-Independent

Inventors: Qian Huang, et al.

murine hsp70.1 -> Translate • 1-frame

DNA sequence 1929 bp ATGGCCAAGAAC ... GAGGTGGATTAG linear

571 /101 AAG GGC GAG CGC AAC GTG CTC ATC TTC GAC CTG G E R N V L I F D L 601/201 531/211 TIGG GGC GGC ACG TTC GAC GTG TCC ATC CTG ACG ATC GAC GAC GGC ATC TTC GAG GTG AAG 661/221 591/231 GOO ACG GOG GGC GAC ACG CAC CTG GGA GGG GAG GAC TTC GAC AAC CGG CTG GTG ACC CAC 'À T A G D T H L G G E D F D N R L V S H 721/241 751/251 THE GTG GAG GAG THE AAG AAG CAE AAG AAG GAS AME AGE CAG AAC AAG CGC GCG GTG F V E E F K R K H K K D I S Q N K R A V 781/261 811/271 CGG CGG CTG CGC ACG GCG TGT GAG ACG GCC AAG ACG ACG CTG TCG TCC AGC ACC CAG GCC R R L R T A C E R A K R T L S S S 841/281 871/291 AGO CTG GAG ATO GAC TOT CTG TTC GAG GGC ATO GAC TTC TAC ACA TOO ATO ACG GGG GCG S L E I D S L F E G I D F Y T S I T R A 931/311 CGG TTC GAA GAG CTG TGC TCG CAC CTG TTC CGC CGC ACG CTG GAG CCC GTG GAG AAG CCC LEPVEKA 961/321 991/331 CTG CGC GAC GCC AAG ATG GAC AAG GCC CAG ATC CAC GAC CTG GTG GTG GGC GGC TCG L R D A K M D K A Q I H D _021/341 1051/351 1051/351 ACG CGC ATC CCC AAG GTG CAG AAG CTG CTG CAG GAC TTC TTC AAC GGG CGC GAC CTG AAC RIPKVQKLLQDFFNG B D L 1091/361 1111/371 AAC AGC ATC, AAC CCG GAC GAG GCG GTG GCC TAC GGG GCG GTG CAG GCG ACC ATC CTG N P D E A V A Y G A A V Q A A I L ह. **5** I 1141/381 1171/391 WIG GGG GAC AAG TOG GAG AAC GTG CAG GAC CTG CTG CTG GAC GTG GCG CCC ENVQDLLLDVAP